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BIOGENIC SYNTHESIS OF COBALT OXIDE NANOPARTICLES USING *Coleus Amboinicus* LEAF EXTRACT AND ITS ANTIBACTERIAL PROPERTIES

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ABSTRACT

The physicochemical methods used for nanoparticle synthesis frequently involve toxic chemicals and generate hazardous by-products. Consequently, the scientific community continues to explore alternative nanoparticle synthesis strategies that are less toxic, environmentally benign, cost-effective, and sustainable. In this context, cobalt oxide nanoparticles are of particular interest owing to their broad spectrum of applications. So, green synthesis methods of these nanoparticles are eco-friendly and could prove an alternative to physical and chemical methods. In this study, we utilise aqueous extract prepared from the leaves of *Coleus Amboinicus* for the synthesis of Co_3O_4 nanoparticles. The crystallite size of synthesised nanoparticles from XRD studies is found to be around 8.378 nm. The biosynthesised nanoparticles are characterised using various analytical techniques, including FTIR, XRD, SEM-EDX, and UV-Visible spectroscopy. The antibacterial activity of the synthesised Co_3O_4 nanoparticles (NPs) was evaluated against a panel of Gram-positive and Gram-negative bacteria. Microbial growth is found to be adversely inhibited by both cobalt nitrate salt solution and Co_3O_4 Nps, with the Co_3O_4 Nps exhibiting notably higher antimicrobial activity compared to the metal salt solution.

Keywords: Green Synthesis**,** *Coleus Amboinicus*, Co_3O_4 Nps, EDX, Antibacterial Activity.
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INTRODUCTION

Nanotechnology involves the manipulation of matter at the molecular level and has firmly established itself across a wide range of applications.[1-6] Recently, cobalt nanoparticles have garnered considerable attention due to their greater economic viability compared to noble metal nanoparticles. Among the commonly used synthetic approaches, chemical methods can efficiently produce nanoparticles with controlled size distribution in a relatively shorter time.[7-9] However, these methods typically require corrosive chemicals, leading to hazardous waste generation and substantial energy consumption. Similarly, physical techniques such as microwave processing and cluster beam deposition require high temperatures, pressures, and radiation. Therefore, the synthesis of nanoparticles has increasingly embraced the principles of "green chemistry," which utilises biological agents like bacteria, fungi, and plants.[10] Nanoparticles have broad applications in medicine, particularly as antimicrobial agents. The fact that growing resistance of bacteria and fungi to conventional medicines has opened a strong drive within the scientific community to develop efficient antimicrobial agents. This study is designed to optimise the use of cobalt oxide nanoparticles, green-synthesised using *Coleus Amboinicus* leaf extract, as antibacterial agents. This synthetic method follows a bottom-up approach, requiring less energy and minimising ingredient waste while ensuring precise control of reagents, leading to a uniform distribution of particles. The properties of the nanoparticles were analysed using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), SEM-EDX analysis, and UV-visible spectroscopy. The antimicrobial potential of the synthesised nanoparticles was also evaluated against some Gram-positive and Gram-negative bacteria and Fungi.

EXPERIMENTAL

The *Coleus Amboinicus* leaves were collected from local areas of Malappuram, Kerala. Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$) was purchased from E Merck. Double-distilled water was used for all experiments. All glassware was thoroughly washed with distilled water and dried in an oven. Mueller Hinton Agar,

Trypticase Soy Broth and Agar powder were purchased from M/s Hi-Media, Mumbai, India. All the pathogenic strains used in this study for assessments of antimicrobial property were previously isolated from clinical samples, biochemically characterised and identified via 16S rRNA sequencing (molecular identification). They were regularly subcultured and maintained on their respective agar media slants at 4°C. The solvents and chemicals used in the study were procured from M/s Merck Chemicals and M/s Hi-Media (Mumbai, India) and were of analytical grade with a purity of ≥ 99%, unless otherwise specified.[12]

Preparation of *Coleus Amboinicus* Leaf Extract

Fresh Coleus Amboinicus leaves were thoroughly washed under running tap water, followed by rinsing with double-distilled water to remove dirt and other contaminants, and then air-dried at room temperature. The dried leaves were ground using a homogeniser. Subsequently, 5 g of the ground material was mixed with 100 ml of double-distilled water and incubated in a water bath at approximately 70 °C for 30 minutes. After cooling, the extract was filtered through Whatman No. 1 filter paper to remove solid residues and stored at 4 °C for further use.

Biosynthesis of Cobalt Oxide Nanoparticles (Co_3O_4 Nps)

The synthesis was performed using Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$) as precursor. 1mM aqueous solution of cobalt nitrate was prepared using double-deionised water. The pH of the leaf extract was adjusted to around 7-8. To 20ml of the extract, 30ml of 1mM cobalt nitrate solution was added dropwise with incubation at 80°C for 30 minutes. The formation of cobalt ions was indicated by a colour change from colourless to light brown.[11] The obtained precipitates were oven-dried at 95°C for 2 hours, finely ground, and analysed using various spectrophotometric techniques.

Characterization Techniques

The Co_3O_4 NPs were characterised by various techniques. The absorption spectra of the Co_3O_4 NPs were studied using UV-Visible spectroscopy (Thermofischer Double Beam UV Visible Spectrophotometer model Genesys 180). Surface functionalisation of *Coleus Amboinicus* leaf extracts on the surface of Co_3O_4 NPs was investigated by FTIR (Schimadzu IR Affinity model 1s double beam spectrometer) with the wave number range of 4000 cm^{-1} to 400 cm^{-1}. The crystalline nature and phase identification were analysed by XRD (Panalytical Xpert 3) method with (λ=1.54 A°) radiation. Surface morphology and particle size of the nanoparticles were examined by the scanning electron microscopy technique (Zeiss MERLIN HR -SEM). The elemental composition of Co_3O_4 Nps was determined by EDX (OXFORD instruments) analysis.

Antibacterial Activity Studies

The synthesised Co_3O_4 Nps were evaluated for their antibacterial activity against different Gram-positive and Gram-negative bacterial strains, including *Bacillus subtilis*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa.* The antimicrobial activity of the samples was determined using the agar-well diffusion method. Samples were tested against Gram-positive bacteria (Bacillus subtilis and Streptococcus pyogenes), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), and the fungus Candida albicans. The inoculum for each strain was prepared by growing them in Trypticase Soy Broth (TSB) at 37°C for 18–24 hours. The culture's turbidity was adjusted to match the 0.5 McFarland Standard by diluting it with a 0.9% NaCl solution.[13] Wells with a diameter of 6 mm were created on Muller-Hinton agar (MHA) plates using a gel puncture tool. The plates were then inoculated by swabbing the microbial pathogens to ensure a uniform lawn of bacterial growth. Each well was filled with 10 µL (10 µg/mL) of the sample and references. Cotton pads dipped in the samples were aseptically cut into uniform pieces and placed on the pathogen-swabbed agar plates. The plates were incubated at 37°C for 18-24 hours to allow the formation of antagonistic zones. The presence of a clear zone around the wells and pads indicated antimicrobial efficacy. The experiment was performed in triplicate to ensure reproducibility.[14]

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

The formation of Co_3O_4 NPs was initially indicated by a colour change in the reaction mixture from light brown to dark brown. The UV spectrum of green synthesised Co_3O_4 NPs displayed absorption bands of

300 nm and 514 nm, confirming nanoparticle formation. According to published literature, these bands can be assigned to the $O_2 \rightarrow Co_2^+$ and $O_2^- \rightarrow Co_3^+$ charge transfer processes.

XRD Analysis

The X-ray diffraction pattern of biosynthesised Co_3O_4 NPs is presented in Fig.-1C. The XRD pattern revealed the pure face-centred cubic spinel phase structure of Co_3O_4 Nps was indicated by diffraction peaks at 31.2°, 37.6°, 44.8°, 55.6°, and 59.8°, which were indexed to the (220), (311), (400), (511) and (440) planes. The diffraction peaks closely match the typical peak pattern for pure Co_3O_4 nanoparticles (JCPDS No. 00-042-1467). The interaction of biomolecules with the cobalt precursor generated cobalt and oxygen nucleation sites, which were brought together by the electron-donating properties of the plant molecules, ultimately leading to the formation of Co_3O_4 nanoparticles. The crystalline size of Co_3O_4 NPs was calculated using Debye Scherrer's equation (D = 0.9 k/ß Cos h). In Scherrer's equation, 0.92 is a constant, k is the wavelength of the X-rays, and ß is the full width is the diffraction angle.[15] The estimated crystalline sizes range from 6.6nm to 9.35 nm, yielding an average particle size of 8.378 nm. The average crystalline size of the Co_3O_4 NPs is presented in Table-1.

Table-1: Average Crystalline Size of Cobalt Oxide Nanoparticle

Peak position (2θ degree)	FWHM (degree)	Crystalline size (nm)
31.008	1.0012	8.59
36.648	0.9345	9.35
44.97	1.3467	6.6
59.78	1.0242	9.34
65.19	1.2287	8.01

FT-IR Analysis

The FT-IR spectra of the biosynthesised Co_3O_4 NPs show an absorption band at 3414.08cm^{-1}, corresponding to 0-H stretching of secondary alcohols, including phenolic compounds. The sharp peak at 1628.16 cm^{-1} confirms the presence of amides. The *Coleus Amboinicus* extract contains various phytochemicals such as terpenoids, alkaloids, phenols, steroids, and flavonoids. Upon adding *Coleus Amboinicus* leaf extract to the cobalt nitrate solution, a noticeable colour change occurred, indicating the formation of Co_3O_4 NPs nanoparticles.

Two prominent peaks at 560.86 cm^{-1} and 659.70 cm^{-1} indicates the formation of Co_3O_4 nanoparticles. Co(III)-O bonds were observed at 659.70cm^{-1}, while Co-O stretching vibrations appeared at 560.86 cm^{-1}. The plant extract contributed amide and carboxylic acid to the cobalt surface, facilitating the reduction, capping, and stabilisation of Co_3O_4 NPs. Peaks at 2373 cm^{-1} and 3414 cm^{-1} correspond to C=O asymmetric vibrations and –OH stretching, respectively, suggesting the presence of adsorbed water molecules and carbon species on the nanoparticle surface. These surface-bound carbon and –OH groups play an important role in the reduction process and contribute to the enhanced activity of the Co_3O_4 nanoparticles.[16-18]

SEM and EDX Analysis

The SEM analysis was used to examine the surface morphology of Co_3O_4 nanoparticles. The SEM images revealed that the nanoparticles are composed of spherical particles with a rough and somewhat agglomerated morphology, likely due to hydrogen bonding with bioactive molecules present in the leaf extract. The secondary metabolites and chemical components in the leaf extract play a crucial role in particle agglomeration by enclosing and stabilising each nanoparticle. The average particle size, as determined from the SEM images, was found to be below 20 nm[19].

The EDX spectrum of cobalt oxide nanoparticles showed characteristic peaks between 0 and 1 keV, and at 6.5 and 8 keV, confirming the presence of cobalt in the samples.[20] The surface morphology of the Co_3O_4 nanoparticles, as observed through SEM, is illustrated in Fig.-1A and B, while Table-2 and Fig.-1D represent the EDX data for the Co_3O_4 nanoparticles.

Table-2: EDX Data of Cobalt Oxide

Element	Weight %	Atomic %
C K	0.00	0.00
O K	65.03	82.75
Cl K	6.90	3.96
K K	15.98	8.32
Ca K	4.89	2.48
Co K	7.20	2.49
Totals	100	100

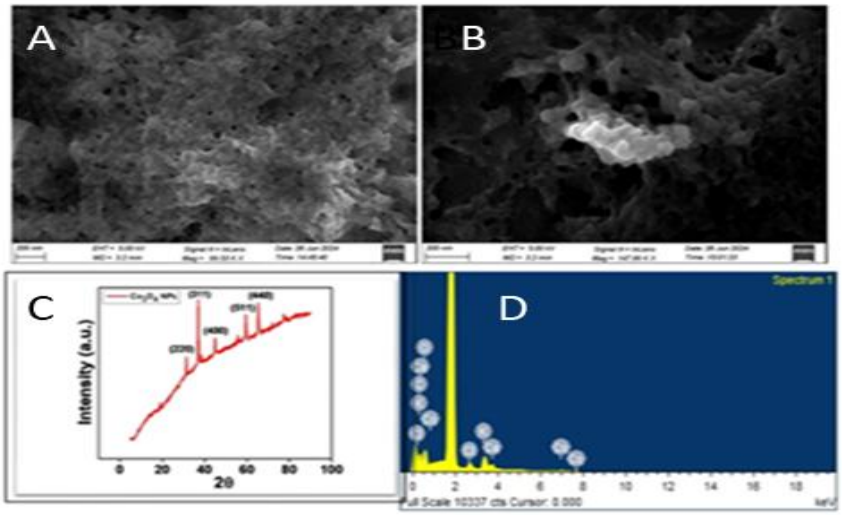

Fig.-1: (A) and (B) SEM Images of Synthesised Cobalt Oxide Nanoparticles, (C) XRD Analysis Image of Cobalt Oxide Nanoparticles, (D) EDX Analysis of Cobalt Oxide Nanoparticles

Antimicrobial Activity

Antibacterial activity of cobalt oxide nanoparticles biosynthesised by *Coleus Amboinicus* leaf extract was determined by agar well diffusion method and presented in Fig.-2. Antibacterial activity of the sample was evaluated against Gram-positive (Bacillus subtilis and Streptococcus pyogenes) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains. The antibacterial activity of the reaction mixture was compared with that of the cobalt nitrate solution after incubation. The antibacterial activity against the test microorganisms was measured by measuring the diameter of the zone of inhibition in each well. The results of the average diameters of inhibition zones are interpreted in Table-3.[21-24]

Table-3: Antimicrobial Activity of Cobalt Oxide Nanoparticle Solution against Various Pathogenic Microorganisms

S.No.	Test pathogen	Co_3O_4 Nps diameter of zone of inhibition	Co metal solution Diameter of zone of inhibition (mm) (D2)	Plant Extract P
1	*Bacillus subtilis*	12 ±± 0.05	10 ±± 0.01	0
2	*Streptococcus pyogenes*	14 ±± 0.10	20 ±± 0.06	0
3	*Escherichia coli*	20 ±± 0.08	15 ±± 0.03	0
4	*Psedumonas aeroginosa*	15 ±± 0.02	13 ±± 0.111	0
5	*Fungi –Candida albicans*	0	0	0

The results show that the reaction mixture containing cobalt oxide nanoparticles showed significant antibacterial activity against the tested microorganisms. The appearance of inhibition zones indicated that biosynthesised cobalt oxide nanoparticles had stronger bactericidal properties against the tested microorganisms than cobalt nitrate metal solution when both treatments were used at the same concentration. A significant activity was given by cobalt oxide NPs against Gram-positive (*Bacillus subtilis, Streptococcus progenes*) with a zone of inhibition of 12 ± 0.05, 14 ± 0.10 mm Gram Gram-negative *(Escherichia coli, Pseudomonas aeruginosa*) with a zone of inhibition of 20 ± 0.08, 15 ± 0.02 mm and *Fungi Candida* albicans with a zero zone of inhibition, respectively.

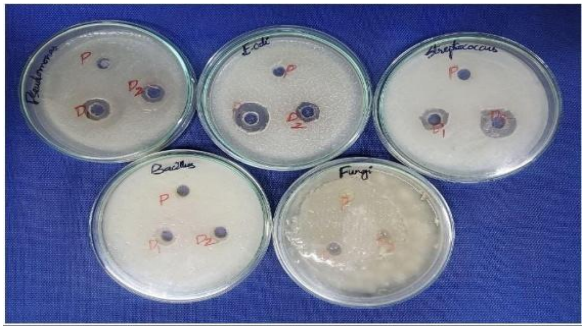

Fig.-2: Antimicrobial Activity of Cobalt Oxide NPs against Gram-positive (*Bacillus subtilis* and *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and fungi *Candida albicans.* (D1: Co_3O_4 Nps, D2: Co metal solution, P: Plant Extract-)

Cobalt nitrate solution showed comparatively less inhibitory activity against all the test pathogens.[12] The antibacterial properties of these nanoparticles are largely attributed to their capacity to generate reactive oxygen species (ROS), which induce oxidative damage to bacterial cell membranes and inhibit bacterial growth by disrupting vital cellular functions. Additionally, the small size of the nanoparticles facilitates their efficient uptake by bacterial cells, ultimately leading to cell damage and death. [25-27]

CONCLUSION

Cobalt oxide nanoparticles were synthesised using a green method with leaf extract from *Coleus Amboinicus*. The prepared nanoparticles were characterized by UV-Visible spectroscopy, FTIR, XRD, and SEM techniques, all of which confirmed the successful formation of cobalt oxide nanoparticles. Their antibacterial activity was evaluated against Gram-positive bacteria (*Bacillus subtilis* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Results indicated that cobalt oxide nanoparticles exhibit higher antibacterial activity compared to their metal solution. Thus, the synthesised nanoparticles show promising potential as antibacterial agents. This study demonstrates that the leaves of Coleus Amboinicus serve as an effective natural source for the green synthesis of cobalt oxide nanoparticles with notable antibacterial activity.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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